Herpes viruses and neuroinflammation

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Chapter 9

Neuroinflammation in schizophrenia related psychosis: a positron emission tomography study

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J Nucl Med 2009; 50: 1801-1807

Abstract

Schizophrenia is a chronic and disabling brain disease characterized by psychotic episodes, with unknown etiology. It is suggested that neuroinflammation plays a role in the pathophysiology of schizophrenia. Neuroinflammation is characterized by the activation of microglia cells, which show an increase in the expression of the peripheral benzodiazepine receptor. The isoquinoline [^{11}C]-*(R)*-PK11195 [*(R)-N*-[^{11}C]-methyl-*N*-(1-methylpropyl)-1-(2-chlorophenyl)isoquinoline-3-carboxamide)] is peripheral benzodiazepine receptor ligand that can be used for imaging of activated microglia cells, and thus neuroinflammation, with positron emission tomography. We hypothesized that neuroinflammation would be more profound in schizophrenic patients during psychosis and it was therefore investigated whether neuroinflammation is present in patients within the schizophrenia-spectrum that were in a psychotic phase.

Seven patients within the schizophrenia spectrum that were recovering from psychosis were included. Recovering psychosis was defined by a score of 5 or more on one item of the positive scale of the PANSS, or a score of 4 on two items. The patients were compared to eight age-matched healthy volunteers. Dynamic PET scans of 60 minutes were acquired after injection of [^{11}C]-*(R)*-PK11195. All subjects underwent a T1- and T2-weighted MRI scan, which were visually examined for abnormalities and used for anatomical coregistration in data-analysis. The PET data was analyzed with a two-tissue compartment model to calculate the binding potential, using the metabolite corrected plasma curve as input.

A significantly higher binding potential of [^{11}C]-*(R)*-PK11195, indicative of neuroinflammation, was found in the hippocampus of schizophrenic patients, when compared to healthy volunteers (2.07 ± 0.42 vs. 1.37 ± 0.30; $p=0.004$). A non-significant 30% higher [^{11}C]-*(R)*-PK11195 binding potential was found in the whole brain grey matter of schizophrenic patients. The MRI images did not reveal any visual abnormalities.

The present study suggests that focal neuroinflammation may play an important role in schizophrenia during psychosis.

Introduction

Schizophrenia is a chronic, disabling brain disease accompanied by psychosis with positive symptoms, such as hallucinations and delusions. Despite considerable research, the exact etiology of psychosis remains unknown. Disturbances in immune mechanisms are thought to play an important role in psychosis of schizophrenia [1]. Although these disturbances in immune mechanisms were mainly found in peripheral blood and in cerebrospinal fluid, they are hypothesized to derive from inflammatory processes in the central nervous system. Indeed, there is evidence from post-mortem studies that schizophrenia is associated with an increased number of activated microglia cells.

Microglia cells are the predominant population of macrophages in the brain and are responsive to injury or infection of brain tissue. In healthy brain tissue, microglia cells have a ramified morphology, characterized by long processes that continuously survey the microenvironment [2]. In response to brain injury or infection, microglia cells change from the ramified morphology into a reactive or amoeboid form. Activated microglia cells, characteristic of neuroinflammation, are involved in the removal of the infectious agents and irreversibly damaged brain tissue. However, in neurological disorders this process runs out of control, resulting in chronic microglia cell activation, which has a detrimental effect. Although neuroinflammation has been shown to play a major role in many neurodegenerative diseases, such as multiple sclerosis, Parkinson's disease and Alzheimer's disease [3], there is only limited and ambiguous data on the presence of neuroinflammation in psychiatric diseases like schizophrenia.

Post-mortem studies in schizophrenic patients have demonstrated the presence of activated microglia cells in the brain. However, the results of these studies are inconsistent. Some studies showed increased density of microglia cells in a subpopulation of schizophrenic patients [4,5], but other studies could not provide evidence for such an increase [6,7]. This might be explained by the differences in markers used for microglia cells and the differences in brain regions that were examined.

Thus far, the majority of the findings supporting the presence of neuroinflammation in schizophrenia are derived from post-mortem studies in a limited number of brain areas without the specific selection of patients with psychosis. Positron emission tomography (PET) provides the opportunity to study the presence of

neuroinflammation in psychotic patients non-invasively. In neuroinflammation, activated microglia cells exhibit an increase in the expression of peripheral benzodiazepine receptors (PBR) in the outer mitochondrial membrane. The PET tracer [^{11}C]-*(R)*-PK11195 (*(R)*-*N*-[^{11}C]-methyl-*N*-(1-methylpropyl)-1-(2-chlorophenyl)isoquinoline-3-carboxamide) is an antagonist of the PBR and binding of [^{11}C]-*(R)*-PK11195 to the PBR can be used to visualize neuroinflammation. [^{11}C]-*(R)*-PK11195 has already been used to show the presence of neuroinflammation in neurological diseases, like Parkinson's disease, Alzheimer's disease, multiple sclerosis and herpes encephalitis (reviewed in [3]. A recent study also showed a general increase in whole brain grey matter binding of [^{11}C]-*(R)*-PK11195 in schizophrenia patients within the first 5 years of disease onset [8]. No specific foci of neuroinflammation were observed. We hypothesized that microglia cells would be more active in schizophrenic patients during psychosis. In the present study, we investigated whether [^{11}C]-*(R)*-PK11195 could demonstrate the presence of neuroinflammation in patients within the schizophrenia-spectrum that were in a psychotic phase.

Material and methods

Subjects

Ten patients were recruited from local psychiatric hospitals based on the following inclusion criteria: 1) fulfilling DSM-IV criteria for the schizophrenia-spectrum (295.xx and 298.xx); 2) psychosis, i.e. a total score of 14 or higher on the positive scale of the positive and negative symptoms scale (PANSS) and at least a score of five on one item or a score of four on two items of the positive scale of the PANSS; 3) age above eighteen; 4) no use of benzodiazepines within 3 half-lives (on average 1-2 weeks) of the benzodiazepines before the start of the study and 5) ability to provide written informed consent. Healthy volunteers, matched for age and gender, were recruited by advertisement and were included if they had 1) no personal history of psychiatric disorders; 2) no family history of psychiatric disorders in their first-degree relatives and 3) no presence of inflammation as measured by C-reactive protein (CRP) (i.e. CRP <0.5 mg/L). Exclusion criteria for all subjects were 1) concomitant or past severe medical conditions; 2) substance abuse; 3) the use of non-steroidal anti-inflammatory drugs (NSAID) or paracetamol; 4) pregnancy and 5) the presence of irremovable magnetic materials in and/or on the body. Classification of diagnoses was performed by an experienced psychiatrist (HCK) using the Schedule for Clinical

Assessment in Neuropsychiatry Version 2 (SCAN2, World Health Organization). Psychopathology in patients was assessed with the PANSS by trained psychiatric nurses.

Of the 20 included subjects, three patients and two healthy volunteers were excluded. One patient withdrew from the study after the MRI scan was performed, so no [^{11}C]-*(R)*-PK11195 PET scan was made. A second patient had too much head movement during the MRI scan and consequently the MRI scan could not be used for normalization of the PET data. A third patient had enlarged ventricles within the physiological range, which hampered normalization of the PET scan. Of two healthy volunteers the PET scan was not performed because the arterial catheter could not be placed. No healthy volunteers were excluded due to elevated CRP.

The study was approved by the medical ethical committee of the University Medical Center Groningen. All subjects provided written informed consent after receiving a complete description of the study.

Radiochemistry

[^{11}C]-*(R)*-PK11195 was labeled by trapping [^{11}C]-methyl iodide in a solution of 1 mg *(R)*-N-desmethyl-PK11195 and 10 mg potassium hydroxide in 300 µl dimethylsulfoxide. The reaction mixture was allowed to react for 1 minute at 40 °C, neutralized with 1M HCl and passed through a 45 µm Millex HV filter. The filtrate was purified by HPLC using a µBondapak C18 column (7.8x300 mm) with acetonitrile/25 mM NaH_2PO_4 (pH 3.5) (55/45) as the eluent (flow 5 ml/min). To remove the organic solvents from the product, the collected HPLC fraction (retention time 7 min) was diluted with 100 ml of water and passed through an Oasis HLB 30 mg (1 cc) cartridge. The cartridge was washed twice with 10 ml of water and subsequently eluted with 1 ml of ethanol and 8 ml of water. The product was sterilized by filtration over a 0.20 µm Millex LG filter. The product was obtained in 36±12% radiochemical yield (n=16). Quality control was performed by HPLC, using a Novapak C18 column (150x3.9 mm) with acetonitrile/25 mM NaH_2PO_4 (pH 3.5) (60/40) as the eluent at a flow of 1 ml/min. The radiochemical purity was always >95% and the specific activity was 89±58 GBq/µmol. No differences were found between the injected dose in healthy volunteers (398±38 MBq) and patients (398±61) (p=0.995). The injected mass was slightly higher in patients as compared to healthy volunteers (1.9±1.5 mg/L vs. 0.7±0.4 mg/L, p=0.051), due to a lower specific activity in patients (56±42 GBq/µmol vs. 112±58 GBq/µmol, p=0.053).

PET protocol

An arterial catheter was inserted in the radial artery after testing for collateral circulation with the Allen test and injection of 1% lidocaine (Fresenius Kabi Nederland BV, 's Hertogenbosch, The Netherlands) for local anesthesia. In the other arm, a venous catheter was inserted in the antebrachial vein. Positron emission tomography imaging was performed with the ECAT EXACT HR+ camera (Siemens, Knoxville, Tenessee). Head movement was minimized with a head-restraining adhesive band and a neuroshield was used to minimize the interference of radiation from the subject's body. A 60-min emission scan in 3D-mode was performed, starting simultaneously with the intravenously injection of [^{11}C]-*(R)*-PK11195. The tracer was injected at a speed of 0.5 ml/sec (total volume of 8.3 ml).

After radiotracer injection, arterial blood radioactivity was continuously monitored with an automated sampling system (Veenstra Instruments, Joure, The Netherlands). Five extra blood samples were collected at 10, 20, 30, 45 and 60 min after [^{11}C]-*(R)*-PK11195 injection to determine the amount of radioactivity in blood and plasma to calibrate the sampling system. The arterial blood samples that were collected at 20, 45 and 60 min after [^{11}C]-*(R)*-PK11195 injection were also used for metabolite analysis. These blood samples were centrifuged at 3000 *g* for 3 min and 1.5 ml plasma was collected. Then 2 ml of acetonitrile (Rathburn Chemicals Ltd, Walkerburn, Scotland) was added to the plasma to precipitate plasma proteins. The plasma samples were shaken on a vortex mixer for 30 s and centrifuged at 3000 *g* for 5 min. A volume of 1 ml of the supernatant was injected into a HPLC system, consisting of a Waters 590 HPLC-pump (Waters Corporation, Milford, USA) and an Alltima 5μm RP-C_{18} column (250x10.0 mm I.D.) (Alltech, Laarne, Belgium). The mobile phase consisted of a mixture of 69.5% of acetonitrile (Rathburn Chemicals Ltd, Walkerburn, Scotland), 30% water (Fresenius Kabi, Sevres, France) and 0.5% of triethylamine (Merck, Haarlem, The Netherlands). The flow-rate was set at 5.0 ml/min and samples were collected at time intervals of 30 s. The collected samples were counted for radioactivity using a gamma-counter (LKB Wallac, Turku, Finland).

MRI

All subjects underwent a magnetic resonance imaging (MRI) of the brain using a 3 Tesla Intera MRI scanner (Philips, Best, The Netherlands) obtaining transaxial 3D-T1 gradient echo (repetition time (RT) 25 ms, echo time (ET) 4.6 ms, field of view (FOV) 256x160x204 mm, matrix 265 x 265, slice thickness 2.0 mm, 160 slices, 1 average, α=

30º), 3D-T1 turbo gradient echo (RT 7.5 ms, ET 3.2 ms, FOV 260x160x232.14 mm, matrix 265 x 265, slice thickness 1.0 mm, 160 slices, 1 average, α= 8º), dual echo (RT 3000 ms, ET 26.7 and 120 ms, inversion time (IT) 2800 ms, FOV 220x150x175.31 mm, matrix 265 x 265, slice thickness 3.0 mm, 50 slices, 1 averages, α= 90º) and T2-FLAIR weighted images (RT 11000 ms, ET 100 ms, IT 2800 ms, FOV 220x150x175.31 mm, matrix 265 x 265, slice thickness 3.0 mm, 50 slices, 2 averages, α= 90º) aligned with the AC-PC (anterior commissure-posterior commissure) plane. The 3D-T1 gradient echo weighted MRI images were used to align to the PET image for normalization. The MRI images of all subjects were visually examined for structural abnormalities and neuroinflammation by an experienced neuroradiologist (JCdeG).

Data-analysis

Attenuation correction was performed with the separate ellipse algorithm. Images were reconstructed by filtered back projection in 21 successive frames of increasing duration (6x 10 sec, 2x 30 sec, 3x 1 min, 2x 2min, 2x 3 min, 3x 5min, 3x 10 min). The first two minutes of the PET scan were summed to create an image that resembles a perfusion image, which was used to align the MRI image to the corresponding PET image using Statistical Parametric Mapping (SPM2) software. The aligned MRI image was normalized to the SPM2 MRI template. The normalization was then applied to each individual frame of the PET image. A limited number of brain regions of interest in the neuroinflammatory process were a-priori selected for comparison between psychotic patients and healthy volunteers. [^{11}C]-*(R)*-PK11195 time-activity curves of the frontal, occipital, temporal and parietal lobe, basal ganglia, hippocampus and cerebellum were created using an automated region of interest (ROI) tool. In addition, time-activity curves of the midbrain and brainstem were created using manually drawn ROIs. The time-activity curves were used for kinetic modeling using software developed in Matlab 7.1 (Mathworks, Natick, Massachusets). The individual delay was calculated to correct for the delay in radioactivity measurements in blood, caused by the distance between the subject and the automated sampling system. Two-tissue compartment modeling (figure 1) was used to calculate the K_1-k_4 using the metabolite corrected plasma curve as an input function, correction for the individual delay and an individually fixed blood volume, which was the median of the blood volume of all areas determined using two-tissue compartment modeling with a free blood volume. The fixed instead of the free blood volume was chosen because it provides a more

stable measurement of K_1-k_4. The binding potential was defined as k_3/k_4 and was calculated for each ROI individually.

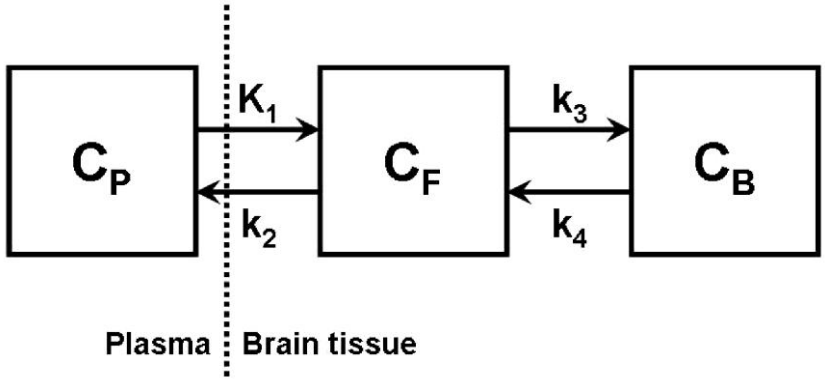

Figure 1 Two-tissue compartment model to describe the kinetics of [^{11}C]-*(R)*-PK11195. Influx of [^{11}C]-*(R)*-PK11195 from plasma (C_P) to brain tissue is described by K_1 and the efflux of from brain tissue to plasma is described by k_2. In brain tissue, [^{11}C]-*(R)*-PK11195 can be either free (C_F) or bound to the peripheral benzodiazepine receptor (C_B). Binding to the receptor is described by k_3 and since [^{11}C]-*(R)*-PK11195 binds reversibly to the PBR, it can return to the free compartment, as described by k_4. The binding potential is defined as k_3/k_4.

Statistics

Statistical analysis was performed with SPSS 16.0. One-way ANOVA was used to determine group differences between the tracer and subject characteristics, and whole brain grey matter binding potential. Statistical analysis on the binding potentials in the examined brain regions was performed using a multivariate general linear model, with the whole brain grey matter binding potential as a covariate to correct for global [^{11}C]-*(R)*-PK11195 uptake. To correct for multiple comparisons, the p-value threshold was defined according to Bonferroni. Therefore, 0.05 was divided by the number of brain regions (n=10) resulting in a significance threshold of 0.005. Correlations between the score on the positive scale of the PANSS and binding potentials were assessed with Pearson's product moment correlation coefficient (r) and were assumed to be significant when $p<0.05$.

Results

Subjects

Subject characteristics are displayed in table 1. The diagnostic SCAN interview confirmed that all patients fit within the DSM-IV criteria of schizophrenia spectrum disorders. Five patients were diagnosed as schizophrenia of the paranoid type, and two patients as having a brief psychotic disorder not otherwise specified. Six patients were scanned within 2 months after onset of a psychotic episode and 1 patient was chronically symptomatic. The number of lifetime psychotic episodes ranged from 1 to 4. The average score on the positive scale of the PANSS was 20±3, on the negative scale 17±5 and on the global scale 37±7. No significant differences were found between the age of patients (31±7) and healthy volunteers (27±6) (p=0.124).

Table 1 Subject characteristics

Subject No.	M/F	Age	DSM-IV	Onset 1st psych	No. of psych	Positive PANSS	Negative PANSS	Global PANSS	Anti-psychotic
Healthy volunteers									
1	F	27	–	–	–	–	–	–	–
2	F	39	–	–	–	–	–	–	–
3	F	23	–	–	–	–	–	–	–
4	M	23	–	–	–	–	–	–	–
5	M	24	–	–	–	–	–	–	–
6	M	22	–	–	–	–	–	–	–
7	M	30	–	–	–	–	–	–	–
8	M	27	–	–	–	–	–	–	–
Patients									
1	M	28	295.30	21	4	20	21	40	Olanzapine
2	M	42	298.90	26	2	20	10	28	Zuclopentixol
3	F	40	295.30	40	1	19	18	37	Olanzapine
4	M	28	295.30	26	1	18	19	42	Haloperidol
5	M	31	295.30	29	1	21	19	37	Risperidone
6	M	28	295.30	20	2	25	20	47	Risperidone
7	M	22	298.90	20	2	15	9	30	Risperidone

M, male; F, female; DSM, diagnostic and statistical manual of mental disorders; Psych, psychoses; PANSS, positive and negative syndrome scale

[11C]-(R)-PK11195 PET

A strong significantly higher [11C]-*(R)*-PK11195 binding potential was found in the hippocampus of patients when compared to healthy volunteers (2.07±0.42 vs. 1.37±0.30; p=0.004) (table 2). The whole brain grey (figure 2) and white matter binding potential of [11C]-*(R)*-PK11195 were respectively 30% higher (1.99±0.64 vs. 1.54±0.41; p=0.122) and 20% higher (2.18±0.70 vs. 1.73±0.51; p=0.180) in patients than in healthy volunteers, but this difference did not reach statistical significance. A non-significant focal increase of [11C]-*(R)*-PK11195 binding potential was found in the midbrain (2.63±0.40 vs. 1.68±0.60; p=0.014), basal ganglia (1.82±0.59 vs. 1.39±0.28; p=0.017), pons (2.85±1.42 vs. 1.54±0.32; p=0.027) and cerebellum (1.45±0.48 vs. 1.11±0.22; p=0.040) of patients when compared to healthy volunteers. No statistically significant differences were found in the influx of [11C]-*(R)*-PK11195 (K_1) in any of the examined brain areas between patients and healthy volunteers. In patients a significantly higher efflux (k_2) of [11C]-*(R)*-PK11195 was found in all examined brain areas when compared to healthy volunteers (p<0.005).

No correlations were found between the total score on the positive, negative and global scale of the PANSS with both the whole brain grey matter and hippocampal binding potential. Age, the total number of psychotic episodes, the onset of the first psychotic episode and the duration of the psychotic episodes were not correlated to the [11C]-*(R)*-PK11195 binding potential.

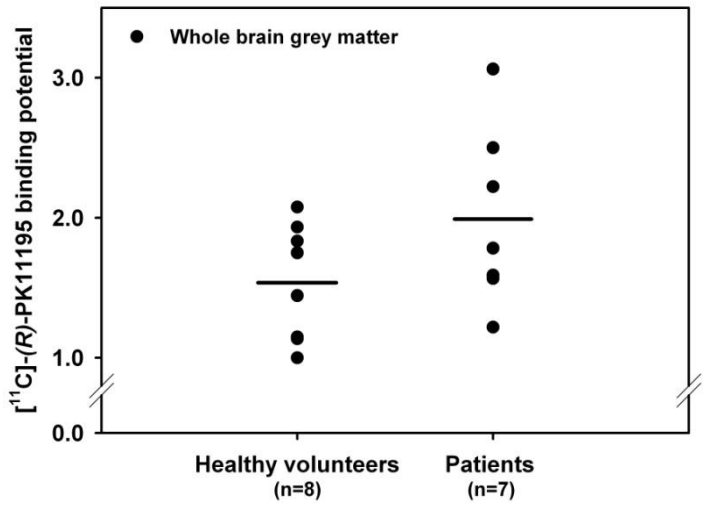

Figure 2 Binding potential of [11C]-*(R)*-PK11195 in the whole brain grey matter. Each dot represents an individual subject and the horizontal lines represent the mean values.

Table 2 Binding potentials of [^{11}C]-*(R)*-PK11195 (average ± standard deviation). Statistical analysis was performed using a multivariate general linear model, with whole brain grey matter as a covariate. *p<0.005

	Healthy volunteers	Patients	p-value
Frontal lobe	1.76 ± 0.75	2.08 ± 0.76	0.459
Occipital lobe	1.83 ± 1.20	1.93 ± 0.74	0.892
Temporal lobe	1.28 ± 0.34	1.64 ± 0.56	0.079
Parietal lobe	1.77 ± 0.93	2.28 ± 1.22	0.720
Basal Ganglia	1.39 ± 0.28	1.82 ± 0.59	0.017
Thalamus	1.61 ± 1.44	1.49 ± 0.35	0.742
Hippocampus	1.37 ± 0.30	2.07 ± 0.42	0.004*
Midbrain	1.68 ± 0.60	2.63 ± 0.40	0.014
Cerebellum	1.11 ± 0.22	1.45 ± 0.48	0.040
Pons	1.54 ± 0.32	2.85 ± 1.42	0.027

MRI

Visual examination of the MRI scans by an experienced neuroradiologist did not show any gross structural abnormalities in either patients or controls. Especially, no white matter lesions, indicative of neuroinflammation, were found.

Discussion

In the present study, we have shown a statistically significant increased binding potential of [^{11}C]-*(R)*-PK11195 in the hippocampus of psychotic patients as compared to healthy volunteers, indicating the presence of neuroinflammation. To our knowledge, this is the first study that reveals the presence of neuroinflammation in a focal area in living patients during psychosis. The presence of focal neuroinflammation in the hippocampus was accompanied by a non-significant increase in the whole brain grey and white matter [^{11}C]-*(R)*-PK11195 binding potential.

In a recent study, Van Berckel *et al.* [8] showed a small increase in whole brain grey matter binding potential of [^{11}C]-*(R)*-PK11195 in stable schizophrenic patients, with no reported focal increase. This is in line with the hypothesis of our study that focal neuroinflammation is a feature of psychosis and not necessarily present in stable schizophrenic patients. The focal neuroinflammation may evolve into a more widespread process in time. Increased focal neuroinflammation during psychosis is an

important pathological finding. The neuroinflammation found in the hippocampus may reflect exceptional vulnerability of this region during psychosis.

The relation between schizophrenia and neuroinflammation was shown in several post-mortem studies, showing an increase in the number of activated microglia cells [4,5,7], but other studies failed to show differences in the presence of activated microglia cells between the brain of schizophrenic patients and control subjects [6,9-11]. Steiner *et al.* [6] could not find support for the presence of activated microglia cells in schizophrenia, but two patients that died from suicide during acute psychosis showed highly elevated microglia cell activation. In a subsequent study, Steiner *et al.* [12] found a significantly increased density of activated microglia cells in the dorsolateral prefrontal cortex, nucleus accumbens and mediodorsal thalamus of six schizophrenic patients that committed suicide during acute psychosis as compared to ten non-suicidal schizophrenic patients. A non-significant increased density of activated microglia cells was found in the hippocampus. No differences in the density of activated microglia cells were found between non-suicidal schizophrenic patients and healthy volunteers. Although suicidal behavior may be linked to other regions of focal neuroinflammation, these results are consistent with the findings in the present study that focal neuroinflammation occurs predominately during psychosis.

Neuroinflammation is characterized by the activation of microglia cells, however, activated astrocytes also play an important role in the neuroinflammatory process [13]. Like in activated microglia cells, the expression of the PBR is also increased in activated astrocytes [14-16]. Increased [^{11}C]-*(R)*-PK11195 can therefore represent activated microglia cells and/or astrocytes. To date, the cell type that is responsible for the increased uptake of [^{11}C]-*(R)*-PK11195, and thus the increased expression of the PBR in neurological disorders, is not exactly known. However, many studies are performed to unravel the contribution of activated microglia cells and/or astrocytes to the PBR mediated [^{11}C]-*(R)*-PK11195 uptake. It has been shown that the contribution is dependent on the phase of activation of microglia cells and astrocytes [14] and on the type of lesion that is investigated. For example, in facial nerve axotomy, only microglia cells were responsible for the [^{11}C]-*(R)*-PK11195 signal [17], whereas in a toxic lesion of the hippocampus an increase in PBR expression was found in both microglia cells and astrocytes [18]. However, it cannot be excluded that the increased [^{11}C]-*(R)*-PK11195 binding potential found in this study is due to activated astrocytes only, unrelated to a neuroinflammatory process. The calcium binding protein S100B, which is primarily expressed by activated astrocytes, was found to be increased in

serum and plasma of schizophrenic patients without damage to neurons and astrocytes [19]. However, since it is not known if S100B expressing astrocytes also show an increase in PBR expression, and since it has been suggested that S100B contributes to neuroinflammation by activation of microglia cells [20], additional research is necessary to unravel the exact mechanism behind S100B expression in schizophrenia. Taken together, regardless of activated microglia cells and/or astrocytes being responsible for the increased expression in the PBR, the focal increased [^{11}C]-*(R)*-PK11195 in schizophrenia most likely represents a neuroinflammatory process.

In the present study, neuroinflammation was found in the hippocampus. Hippocampal pathology in schizophrenia is abundantly reported in the literature. MRI studies showed a decrease in hippocampal size in schizophrenic patients, even in prodromal and first-episode patients showing that it is not secondary to treatment [21,22]. Despite the reported decrease in hippocampal volume, there is no evidence for a change in the total number of neurons in the hippocampus of schizophrenia [23,24]. More likely, the morphology of the hippocampal neurons in schizophrenia is altered with respect to parameters such as size, shape and organization [25]. The involvement of microglia cells in hippocampal pathology is hitherto unknown.

The marked increase in [^{11}C]-*(R)*-PK11195 binding potential in the hippocampus suggests that neuroinflammation plays an important role in schizophrenia, especially during psychosis, and provides a potential target for therapy. In fact, it was shown that both patients with an acute exacerbation of schizophrenia [26] and chronic schizophrenic patients [27] that were treated with the cyclooxygenase-2 inhibitor celecoxib in addition to treatment with risperidone, had a significantly greater improvement on the PANSS than the patients that were treated with risperidone alone. In addition, the broad spectrum tetracycline antibiotic minocycline, was found to be beneficial as an add-on treatment in schizophrenia, in both an open label study [28] and a double-blind placebo controlled study [29]. Besides celecoxib and mincocycline, addition of aspirin to regular antipsychotic treatment over a period of three months was also found to substantially reduce the symptoms of schizophrenia when compared to patients that were treated with antipsychotics only [30]. Thus the anti-inflammatory drugs celecoxib, minocycline and aspirin can improve the symptoms of schizophrenia, most likely due to inhibition of pro-inflammatory cytokines. These results are in agreement with the presence of a neuroinflammatory process in schizophrenia.

Because all patients were taking antipsychotic medication during the period in which the [^{11}C]-*(R)*-PK11195 PET scan was performed, there is a possibility that the medication influenced the microglia cell activation. *In vitro* studies on the effect of antipsychotic drugs on microglia cells show that they decrease the neurotoxic molecules released by activated microglia cells. Both risperidone and haloperidol were found to inhibit the production of nitric oxide, the expression of inducible nitric oxide synthase and the production of pro-inflammatory cytokines by microglia cells treated with interferon-γ [31]. Olanzapine was also found to have anti-inflammatory properties, since the production of nitric oxide by lipopolysaccharide-stimulated microglia cells was reduced by pre-treatment with olanzapine [32]. In addition to the *in vitro* studies, we have recently shown that both clozapine and risperidone inhibited the activation of microglia cells in a rat model of herpes simplex encephalitis, while chronic treatment with clozapine and risperidone of healthy rats did not cause activation of microglia cells (data will be published elsewhere). Thus antipsychotic drugs most likely affect neuroinflammation in such a way that they decrease the microglia cell activation rather than cause activation of the microglia cells.

In addition to antipsychotics, all patients have used benzodiazepines. Because benzodiazepines were found to have affinity for the PBR, the patients had to stop with the use of benzodiazepines at minimal 3 half-lives (on average 1-2 weeks) of the benzodiazepines before the start of the study, to prevent a direct effect on the binding of [^{11}C]-*(R)*-PK11195. It can, however, not been ruled out that treatment with benzodiazepines affected the expression of the PBR. Most studies on benzodiazepines and other PBR ligands, such as Ro5-4864 and PK11195, indicate a neuroprotective effects of these ligands [33]. Wilms *et al.* [34] have shown that midazolam, clonazepam and diazepam interfered with the *in vitro* synthesis and release of pro-inflammatory cytokines by microglia cells. *In vivo*, it has been shown that diazepam protected against neuronal death in the hippocampus after transient forebrain ischemia [35]. Benzodiazepines, like antipsychotic, thus rather decreased neuroinflammation then being responsible for the increased [^{11}C]-*(R)*-PK11195 binding potential in the hippocampus. However, additional research on the role of both antipsychotics and benzodiazepines is necessary to determine their (protective) role in neuroinflammation.

Conclusion

The present study in patients within the schizophrenia spectrum suggests the presence of focal neuroinflammation in the hippocampus, during psychosis. This focal neuroinflammation was not accompanied by structural abnormalities. Which cell type is responsible for the increased [^{11}C]-*(R)*-PK11195 binding potential and how neuroinflammation is related to psychosis in schizophrenia, remains to be elucidated. Therefore, future studies are needed for further evaluation of the role of focal neuroinflammation during psychosis and the feasibility of anti-inflammatory drugs to treat the disease.

Acknowledgement

We would like to thank Rixt Elgersma and the nurse staff of Lentis Winschoten, The Netherlands for accompanying the patients. This study was funded by the Stanley Medical Research Institute, Grant-ID 05-NV-001.

References

1 Muller N, Schwarz MJ. A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders. Eur.Arch.Psychiatry Clin.Neurosci. 2008; 258:97-106

2 Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308:1314-1318

3 Doorduin J, de Vries EFJ, Dierckx RA, Klein HC. PET imaging of the peripheral benzodiazepine receptor: monitoring disease progression and therapy response in neurodegenerative disorders. Curr.Pharm.Des 2008; 14:3297-3315

4 Bayer TA, Buslei R, Havas L, Falkai P. Evidence for activation of microglia in patients with psychiatric illnesses. Neurosci.Lett. 1999; 271:126-128

5 Radewicz K, Garey LJ, Gentleman SM, Reynolds R. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. J.Neuropathol.Exp.Neurol. 2000; 59:137-150

6 Steiner J, Mawrin C, Ziegeler A, et al. Distribution of HLA-DR-positive microglia in schizophrenia reflects impaired cerebral lateralization. Acta Neuropathol. 2006; 112:305-316

7 Wierzba-Bobrowicz T, Lewandowska E, Lechowicz W, Stepien T, Pasennik E. Quantitative analysis of activated microglia, ramified and damage of processes in the frontal and temporal lobes of chronic schizophrenics. Folia Neuropathol. 2005; 43:81-89

8 van Berckel BN, Bossong MG, Boellaard R, et al. Microglia Activation in Recent-Onset Schizophrenia: A Quantitative (R)-[(11)C]PK11195 Positron Emission Tomography Study. Biol.Psychiatry 2008; 64:820-822

9 Arnold SE, Trojanowski JQ, Gur RE, Blackwell P, Han LY, Choi C. Absence of neurodegeneration and neural injury in the cerebral cortex in a sample of elderly patients with schizophrenia. Arch.Gen.Psychiatry 1998; 55:225-232

10 Falke E, Han LY, Arnold SE. Absence of neurodegeneration in the thalamus and caudate of elderly patients with schizophrenia. Psychiatry Res. 2000; 93:103-110

11 Kurumaji A, Wakai T, Toru M. Decreases in peripheral-type benzodiazepine receptors in postmortem brains of chronic schizophrenics. J.Neural Transm. 1997; 104:1361-1370

12 Steiner J, Bielau H, Brisch R, et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. J.Psychiatr.Res. 2008; 42:151-157

13 Chavarria A, Alcocer-Varela J. Is damage in central nervous system due to inflammation? Autoimmun.Rev. 2004; 3:251-260

14 Chen MK, Guilarte TR. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. Pharmacol.Ther. 2008; 118:1-17

15 Itzhak Y, Baker L, Norenberg MD. Characterization of the peripheral-type benzodiazepine receptors in cultured astrocytes: evidence for multiplicity. Glia 1993; 9:211-218
16 Raghavendra Rao VL, Dogan A, Bowen KK, Dempsey RJ. Traumatic brain injury leads to increased expression of peripheral-type benzodiazepine receptors, neuronal death, and activation of astrocytes and microglia in rat thalamus. Exp.Neurol. 2000; 161:102-114
17 Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine')--binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. J.Neurocytol. 1997; 26:77-82
18 Kuhlmann AC, Guilarte TR. Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity. J.Neurochem. 2000; 74:1694-1704
19 Steiner J, Bielau H, Bernstein HG, Bogerts B, Wunderlich MT. Increased cerebrospinal fluid and serum levels of S100B in first-onset schizophrenia are not related to a degenerative release of glial fibrillar acidic protein, myelin basic protein and neurone-specific enolase from glia or neurones. J.Neurol.Neurosurg.Psychiatry 2006; 77:1284-1287
20 Rothermundt M, Peters M, Prehn JH, Arolt V. S100B in brain damage and neurodegeneration. Microsc.Res.Tech. 2003; 60:614-632
21 Pantelis C, Velakoulis D, McGorry PD, et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 2003; 361:281-288
22 Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET. Meta-analysis of regional brain volumes in schizophrenia. Am.J.Psychiatry 2000; 157:16-25
23 Heckers S, Heinsen H, Geiger B, Beckmann H. Hippocampal neuron number in schizophrenia. A stereological study. Arch.Gen.Psychiatry 1991; 48:1002-1008
24 Walker MA, Highley JR, Esiri MM, et al. Estimated neuronal populations and volumes of the hippocampus and its subfields in schizophrenia. Am.J.Psychiatry 2002; 159:821-828
25 Harrison PJ. The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology (Berl) 2004; 174:151-162
26 Muller N, Riedel M, Scheppach C, et al. Beneficial antipsychotic effects of celecoxib add-on therapy compared to risperidone alone in schizophrenia. Am.J.Psychiatry 2002; 159:1029-1034
27 Akhondzadeh S, Tabatabaee M, Amini H, Ahmadi Abhari SA, Abbasi SH, Behnam B. Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. Schizophr.Res. 2007; 90:179-185

28 Miyaoka T, Yasukawa R, Yasuda H, Hayashida M, Inagaki T, Horiguchi J. Minocycline as adjunctive therapy for schizophrenia: an open-label study. Clin.Neuropharmacol. 2008; 31:287-292

29 Levkovitz Y, Mendlovic S, Issaki S, Braw Y, Levkovitch-Verbin H, Gal G. Minocycline ameliorates negative symptoms and cognitive deficits in early-phase schizophrenia: a double-blind, randomized placebo-controlled study. Schizophr.Res. 2008; 98 (suppl 1):14-

30 Laan W. Anti-inflammatory drugs and psychosis.: University of Utrecht, 2008

31 Kato T, Monji A, Hashioka S, Kanba S. Risperidone significantly inhibits interferon-gamma-induced microglial activation in vitro. Schizophr.Res. 2007; 92:108-115

32 Hou Y, Wu CF, Yang JY, et al. Effects of clozapine, olanzapine and haloperidol on nitric oxide production by lipopolysaccharide-activated N9 cells. Prog.Neuropsychopharmacol.Biol.Psychiatry 2006; 30:1523-1528

33 Papadopoulos V, Lecanu L. Translocator protein (18 kDa) TSPO: An emerging therapeutic target in neurotrauma. Exp.Neurol. 2009;

34 Wilms H, Claasen J, Rohl C, Sievers J, Deuschl G, Lucius R. Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: evidence from activated microglial cells in vitro. Neurobiol.Dis. 2003; 14:417-424

35 Sarnowska A, Beresewicz M, Zablocka B, Domanska-Janik K. Diazepam neuroprotection in excitotoxic and oxidative stress involves a mitochondrial mechanism additional to the GABAAR and hypothermic effects. Neurochem.Int. 2009; 55:164-173